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Alkali-Catalyzed Organosolv Pretreatment of Lignocellulose Enhances Enzymatic Hydrolysis and Results in Highly Antioxidative Lignin

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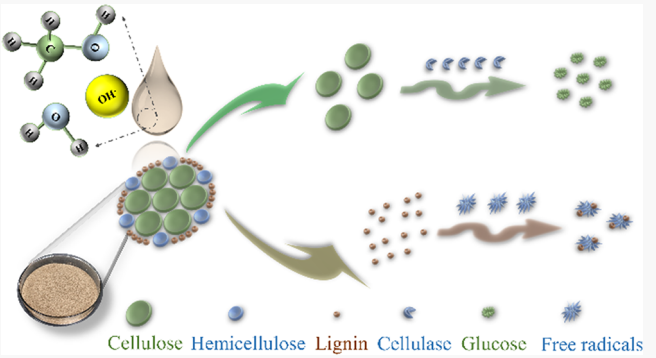
ABSTRACT: Herein, efficient exploitation of biomass with diluted alkali-aided methanol organosolv treatment has been proposed. The ultrahigh glucose yield and high value-added lignin from xylose residue were simultaneously achieved via the mild alkali catalytic organosolv treatment and enzymatic hydrolysis. The NaOH-aided methanol pretreatment resulted in a more efficient delignification of 86.7%, which significantly facilitated the cellulase accessibility toward gaining a high glucose yield of 97.7%. In addition, the recovered lignin is highly pure with small and homogeneous molecular weight and high thermal stability. The total phenolic and antioxidant activities of the lignin were carried out by a Folin−Ciocalteu method and radical DPPH and ABTS assays, respectively. Results revealed that the modified lignin effectively scavenged radicals compared to the commercial antioxidants (butylated hydroxytoluene). Overall, this study provides a mild but a highly promising pretreatment approach for energy production from biomass. The excellent radical scavenging capacity of the obtained lignin further sets the stage for biobased functional materials in the popular biorefinery.



1. INTRODUCTION

The burgeoning challenges such as petroleum-derived energy shortage along with environmental degradation namely destruction of ecosystem, habitat eradication, and extinction of wildlife have paved the way for biobased products from lignocellulose biomass and believed to provide a sustainable solution for the current and future needs.[1,2] In the present bioeconomy, it is not feasible to obtain high-value products from lignocellulose at a large scale. In order to increase the efficiency, pretreatment of lignocellulose is critical, mainly to wane the recalcitrant complex structure.[3]

During the last decade, several protocols have been designed and developed.[4−6] In this set, organosolv pretreatment is useful for successful delignification and efficient separation of cellulose along with obtaining pure lignin.[7] Toward this, organic solvents including low-boiling-point solvents and high-boiling-point solvents, as well as organic acids, ketones, and others are being explored.[8] In addition, harsh reaction conditions such as high temperatures (150−200 °C) are needed, which, certainly, limit the large-scale processes and industrialization.[9] In order to deconstruct the intact lignocellulose structure in a relatively easy process, pretreatment with acid-aided organic solvents such as $FeCl_3$, H_2SO_4, and HCl has gained credibility.[10,11] However, these acidic catalysts could degrade glucose and xylose along with forming complex humins and irreversible lignin condensation.[12] In this regard, alkali-aided organosolv pretreatment could be helpful with subtle cellulose loss and hemicellulose degradation but, more importantly, with high-quality lignin yield.[13,14] For example, hydrolysis/fermentation of oil palm fronds (OPFs) by NaOH-catalyzed ethanol pretreatment with ultrasonic assistance yielded around 20.1, 11.3, and 9.3 g/L of glucose, xylose, and bioethanol, respectively.[15] Similarly, alkaline-catalyzed acetone-based organosolv of a rice straw resulted in a high fermentable glucose of 913 mg/g.[14] Likewise, the total reducing sugar of a cauliflower waste reached a maximum output of 831 mg/g through enzymatic hydrolysis coupled with sodium acetate-aided methanol.[16] In addition, high-purity and antioxidative lignin from a willow could be extracted through a NaOH-catalyzed sulfolane/water system.[17] These successful efforts are being continued with an emphasis on optimizing lignin isolation and/or improving the yield of fermented sugar. However, fractionation for enzymatic hydrol-

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ysis of cellulose and isolation of high-quality lignin under mild alkali-aided organosolv conditions has not yet been explored. It has been established that shedding lignin from lignocellulose will relax the cell wall structure and thus cellulose would be more accessible to enzymes.[18] Furthermore, during the pretreatment process, most of the lignin gets dissolved in the liquor. In this regard, effective lignin recovery needs to be integrated into the biorefinery process, mainly to take advantage of lignin's invaluable applications as a filler, additive, surfactant, and anticancer drug carrier,[19] to name a few. Herein, fractionation of xylose residue components coupled with mild alkali-aided organosolv conditions has been demonstrated as a viable technique to not only digest cellulose easily but also to extract high-quality lignin.

Synthetic molecules such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and propyl gallate (PG) are being used as antioxidants. Naturally available lignin, however, could be a favorable alternative due to lignin's intrinsic polyphenols.[20−22] A couple of successful examples includes lignin-polycaprolactone copolymers with good antioxidant properties[23] and lignin nanoparticles with higher antioxidant activity (RSI of ca. 82) than BHT and BHA.[21] The versatility of the complex molecular structure of lignin is dependent on the isolation process that in turn influences the antioxidant activity. However, lignin isolated through alkali-aided methanol pretreatment has not yet been explored as an antioxidant agent.

The byproduct of xylose production is rich in cellulose and lignin.[1] The fractionation of xylose residue is also being exploited for synthesizing cellulose-based products.[24,25] In this regard, the main research objective of our investigation was to detach cellulose and lignin from the xylose residue through a mild and economical separation process—by treating with a methanol/water solvent system at different reaction temperatures, times, and alkaline concentrations. The obtained cellulose was further subjected to enzymatic hydrolysis to produce glucose so as to cater the bioethanol conversion strategies. In addition, lignin was recycled, and its detailed physicochemical structures and antioxidant activity were elaborated. The outcome believed to open up a new window of opportunities that are environmentally feasible and economical to convert the lignocellulosic biomass and biomass-derived wastes to bioenergy and bioproducts.

2. MATERIALS AND METHODS

2.1. Materials. Xylose residue, produced in the process of xylose production from Corncob by dilute acid hydrolysis, was provided by the Shandong Longlive Corporation (Yucheng, China). Prior to the pretreatment, the xylose residue was washed to near neutral (pH = 6.2) and sieved to a 40−60 mesh flour and then dried at 80 °C to constant weight. It was then extracted with toluene/ethanol (2/1, v/v) for 6 h in a Soxhlet extractor. The chemical compositions of xylose residue (w/w) were 57.5% glucan, 5.7% xylan, 1.6% arabinan, and 21.1% lignin.[26] The cellulase with a filter paper activity of 110 FPU/g was supplied by Shanghai Youtell Biochemical., Ltd. (Shanghai, China). Methanol and sodium hydroxide were purchased from Macklin Biochemical Technology Co., Ltd. (Shanghai, China). All used chemicals were of analytical grade.

2.2. NaOH-Catalyzed Organosolv Pretreatment. The procedure for NaOH-aided methanol extraction of lignin and cellulose from xylose residue is presented here. Briefly, extractive-free xylose residue (2.0 g) was loaded in a 75 mL pressure bottle reactor, together with methanol/water (40 mL, 65/35, v/v) containing 0.25−2.0 wt % NaOH. The processing conditions were: raising from room temperature to desired temperature (20−100 °C) and holding for a preset time (0.5−1.5 h) with magnetic stirring at 300 rpm. Later, the cooled mixture was vacuum-filtered by a G2 filter. The obtained solid phase was washed with 200 mL of deionized water. The neutralized solid was dried at 80 °C before saccharification and coded as S. The dark liquid was poured into vacuum rotary evaporation at 35 °C to recover methanol. The concentrated liquid was added dropwise into an acidic deionized aqueous solution (500 mL, pH = 2, acidified with concentrated hydrochloric acid) with constant stirring. The lignins were then precipitated and recovered by centrifugation at 8000 rpm for 3 min. They were washed with fresh water, freeze-dried, and coded as L.

2.3. Enzymatic Hydrolysis. Enzymatic hydrolysis experiments of the solid residues were conducted at 50 °C in a 25 mL sealed Erlenmeyer flask. The biomass samples of 0.3 g, 15 mL of acetate buffer (pH 4.8, 0.05 M), and cellulase (20 FPU/g substrate) were mixed in a rotary shaker at 150 rpm for 72 h. A solution of 0.3 mL was taken at time intervals 1, 2, 3, 6, 12, 24, 36, 48, 60, and 72 h, and the released glucose was measured according to the literature protocol.[27] Briefly, the collected liquid samples were placed in a boiling water bath for 5 min to terminate the hydrolysis reaction. Later, they were diluted twice with ultrapure water, passed through a 0.22 μm filter membrane, and tested for the sugar content by high-performance liquid chromatography (HPLC).

2.4. Characterization of Pretreated Residues and Lignins. *2.4.1. Characterization of Pretreated Residues.* The compositional analysis of pretreated residues was conducted according to the standard National Renewable Energy Laboratory (NREL) procedures.[26] Fourier transform infrared (FTIR) spectra of fractions were recorded with a Bruker Alpha FTIR spectrophotometer. The data were obtained in the range from 4000 to 400 cm^{-1} at a resolution of 4 cm^{-1} with 32 scans using a KBr tablet method.[28] The crystallinity indexes (CrI) of the cellulose residues were analyzed using a Bruker D8 Advance. The X-ray tube was operated at 40 kV and 50 mA. The intensity data were collected using a continuous scan mode in a 2θ range from 5 to 50° at a scan rate of 0.6°/s. Surface morphology of the cellulose residue was examined by field emission scanning electron microscopy (FESEM) using an EM-30 Plus microscope (Coxem, Korea) at 20 kV acceleration voltage.

2.4.2. Characterization of Lignins. The contents of carbohydrate impurities in the lignin samples were verified according to a literature protocol.[29] Briefly, 10 mg of sample was added to 2.95 mL of acidic solution (6% H_2SO_4) and hydrolyzed at 105 °C for 2.5 h. Subsequently, the liquid sample was diluted 50 times, and the sugar content was analyzed by ion chromatography (IC). Gel permeation chromatography (GPC) analyses of the acylated lignin fractions were determined by a Waters 2695 with a UV detector equipping a 127 PLgel Mixed-E column, which was operated at 40 °C and eluted at a flow rate of 0.6 mL/min.[30] A series of monodispersed polystyrene standards was prepared for generating the calibration curve. Thermogravimetric analysis (TGA) was carried out with a simultaneous thermal analyzer (Netzsch STA 449 F3, Germany) at a heating rate of 10 °C/min under a nitrogen atmosphere.[31] The 2D heteronuclear single quantum coherence (HSQC) NMR spectra of acetylated lignin fractions were recorded by a 400 MHz NMR spectrometer (Avance III, Bruker).[32]

2.5. Antioxidant Activity of Lignins. *2.5.1. Total Polyphenol Content.* The total polyphenol content (TPC) of lignin fractions was determined by Folin−Ciocalteau (F−C) assay.[33] In order to determine the concentration of gallic acid equivalents (c_{GAE}) in the lignin samples, the calibration curve of gallic acid in DMSO was developed with six concentrations ranging from 30 to 600 mg/L. The lignin sample (2 mg/mL) in DMSO was tested for the total polyphenol content. The detailed procedure is as follows: each sample (0.2 mL) was mixed with 0.5 mL of F−C reagent. After 5 min, the Na_2CO_3 solution (1 mL, 15% (w/v)) was added into the obtained mixture, and the total volume was kept to 10 mL with deionized water. The mixture was allowed to stand for 1 h at room temperature in the dark before the UV−vis spectra measurement. The absorbance was recorded at 765 nm (UV−vis) against the reagent blank. The TPC was expressed as the percentage of gallic acid equivalents (GAE, %) in the dried lignin samples.

2.5.2. ABTS Free Radical Scavenging Activity. The antioxidant power (AOP) of lignin was determined by diammonium 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonate) (ABTS) free radical scaveng-

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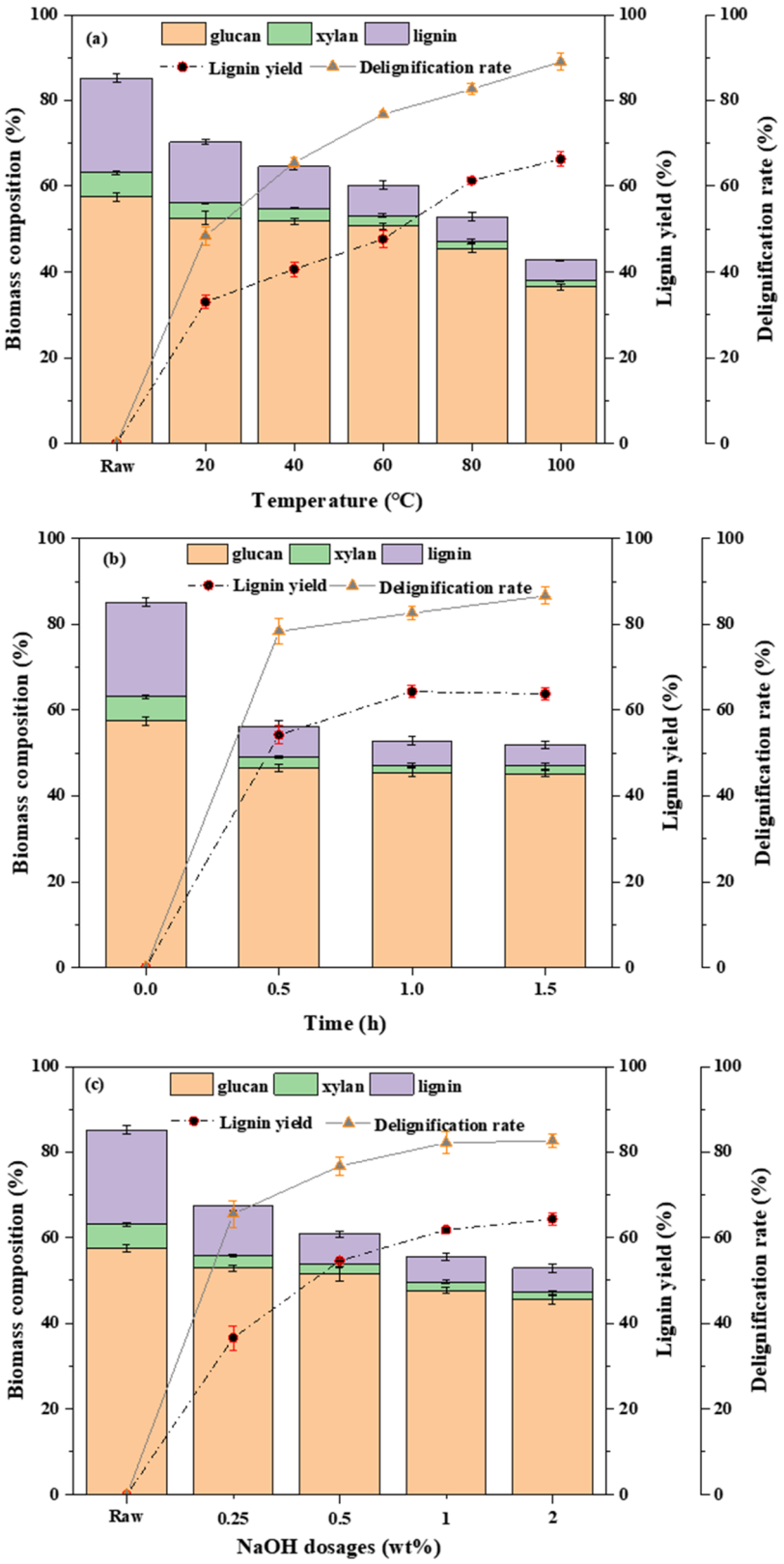

Figure 1. Chemical composition of alkali-aided methanol systems with pretreated xylose residue at different conditions: (a) effect of reaction temperature on pretreatment (reaction for 1 h with 2 wt % NaOH), (b) effect of reaction time on pretreatment (reaction at 80 °C with 2 wt % NaOH), and (c) effect of NaOH dosages on pretreatment (reaction at 80 °C for 1 h).

ing assay.[34] Briefly, the solution formed by 7 mM ABTS and 2.45 mM potassium persulfate at room temperature in the dark for 14 h was adjusted to an absorbance of 0.70 ± 0.02 at 734 nm by diluting 25−35 times with 50% ethanol solution, and the resulting solution was an ABTS free radical solution. A mixture of lignin solution (40 μL, 2 g/L in DMSO) and diluted free radical solution (4 mL) after 6 min was

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detected at 734 nm against the 50% ethanol solution. The blank contains DMSO instead of the lignin solution and a sample with a diluted free radical solution.

2.5.3. DPPH Free Radical Scavenging Activity. The antioxidant power was estimated as the ability of lignin samples to scavenge the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical.[17] The antioxidant activity was displayed as the radical scavenging index (RSI). The IC50 was defined as the lignin concentration with 50% DPPH activity.

3. RESULTS AND DISCUSSION

3.1. Optimization of Methanol-Based Solvent Treatment. Methanol is a low-priced, low-toxicity, water-soluble, and low-boiling-point solvent. It is used routinely and effectively for delignification without catalysts, and its lignin solubility is higher than ethanol.[8] Previously reported pretreatment of a methanol/water (80/20, v/v) system without catalysts focused on the milled wood powder at 220 °C for 30 min, which resulted in 45.5% recovery of lignin in organic fractions.[35] Certainly, these processing conditions need to be improved from the point of view of lignin yield and energy consumption. In this regard, herein, the xylose residue has been treated with the alkali-catalyzed methanol/water system, mainly to reduce the recalcitrance nature of biomass under mild experimental conditions. The changes of chemical composition of the untreated and pretreated xylose residue at various temperatures, times, and alkali dosages are portrayed in Figure 1.

The effect of temperature on the pretreated samples in a 2% NaOH-aided methanol/water system for 1 h is shown in Figure 1a. The yield of recovered cellulose residue was over 54%. The amount of glucan preserved was over 80%, and with an increase in the pretreatment temperature from 20 to 80 °C, the delignification efficiency is found to increase from 48.4 to 82.7%. Interestingly, nearly 50% of lignin has been removed of which 33% was obtained at a low temperature of 20 °C and nearly twice the amount at 80 °C. Further increase to 100 °C wilts the glucan content (~60%) but with a subtle rise in the removal and yield of lignin. Hence, a reaction temperature of 80 °C has been selected as the optimal temperature for the conversion of xylose residue.

Figure 1b depicts the effect of reaction time on the pretreated xylose residue in a 2% NaOH-aided methanol/water system at 80 °C. The yields of recovered cellulose residue were 65.1, 62.5, and 59.8% with extraction times of 0.5, 1, and 1.5 h, respectively. These have been referred to as $S_{A2\text{-}0.5h}$, $S_{A2\text{-}1h}$, and $S_{A2\text{-}1.5h}$, respectively, for brevity, in the rest of the discussion. There was no significant change in the preserved glucan content (83.3 to 77.9%) with increase in the pretreatment time. Lignin was removed effectively (78.4 to 86.7%) with extraction times of 0.5, 1, and 1.5 h, and the corresponding yields are 54.2, 64.3, and 63.8%, respectively, which are labeled as $L_{A2\text{-}0.5h}$, $L_{A2\text{-}1h}$, and $L_{A2\text{-}1.5h}$, respectively. The yield at 1 h is nearly 10% higher than that at 0.5 h. However, at 1.5 h a subtle drop is noticed, presumably due to the decomposition of unstable lignin into smaller fragments. Thus, 1 h has been selected as the optimal time for the conversion of xylose residue.

The influence of alkali dosage on the pretreated xylose residue in a methanol/water system was evaluated at 80 °C for 1 h. As shown in Figure 1c, the recovery yields of cellulose residues are 78.9, 73.2, 66.3, and 62.5% at alkali doses of 0.25, 0.5, 1, and 2%, respectively, and labeled as $S_{A0.25\text{-}1h}$, $S_{A0.5\text{-}1h}$, $S_{A1\text{-}1h}$, and S_{A2-1h}, respectively. It is quite evident that the dissolved amount of lignin increases from 65.6 to 82.7% with a concomitant rise in the glucan content from 79.1 to 92.0%. The recovered lignin yields are found to be 36.6, 54.6, 61.8, and 64.3% labeled as $L_{A0.25\text{-}1h}$, $L_{A0.5\text{-}1h}$, $L_{A1\text{-}1h}$, and L_{A2-1h}, respectively. The delignification rate and lignin yield are stable above 1% alkali dose. Thus, it appears that mild alkali conditions, adopted in this research, are sufficient to break the alkali-sensitive bonds among the lignin and carbohydrate and appear to be quite suitable to deconstruct the biomass. Ironically, excessive alkali amounts destroy cellulose and degrade lignin.[36,37]

3.2. Characteristics of Cellulose Residues. *3.2.1. FTIR Analysis.* The FTIR spectra of a raw material (Raw) and pretreated cellulose residues (S) are shown in Figure 2. The bands were assigned based on the literature report.[38] The strong absorption bands at 3421 and 2905 cm^{-1} were attributed to the OH group stretching and C−H stretching, respectively. The weak signals at 1717 cm^{-1} (carboxylic acid and ester bond in hemicellulose), 1511 cm^{-1} (aromatic skeletal vibration in lignin), and disappeared peak at 1258 cm^{-1} (typical of lignin unit) in the pretreated cellulose residue confirm the removal of hemicellulose and lignin. The intense absorptions at 1430, 1373, 1318, and 1162 cm^{-1} are characteristic of C−O antisymmetric stretching and C−H wagging in the cellulose and accentuate the preservation of amorphous cellulose and crystalline cellulose. Overall, there are no new peaks, or the characteristic cellulose peaks did not disappear, suggesting the intact cellulose structure and the conducive nature of cellulose-rich residue to the enzymatic hydrolysis.

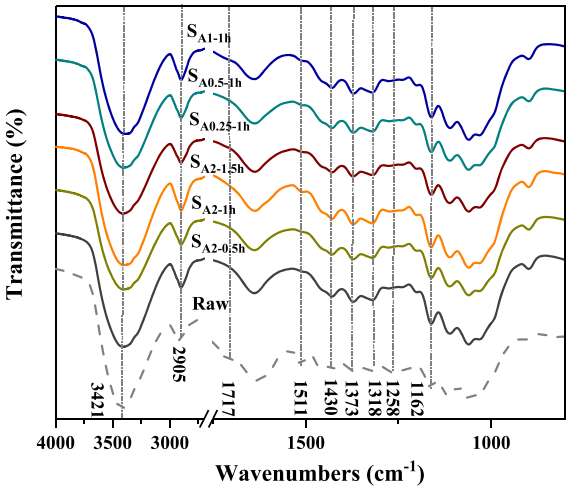

Figure 2. FTIR spectra of the raw material (Raw) and the extracted cellulose residue (S) at different methanol pretreatments.

3.2.2. XRD Analysis. The NaOH-aided methanol solvent pretreatment resulted in the removal of abundant lignin but retained considerable cellulose. In order to assess the cellulose amount, the crystallinity index (CrI) of raw material (Raw) and the extracted cellulose fractions (S) were calculated from the X-ray powder diffraction patterns (Figure 3). The diffraction peaks of crystalline cellulose are seen at 2θ of 16.8 and 22.5°, and the amorphous cellulose at 2θ of 18.6° (Figure 3). The CrI of the Raw is found to be 47.9% with around 9% increase for cellulose fractions (S). The gain could be due to dissolved hemicellulose in the pretreatment solution.[39] The CrI of the S increases with the treatment time and the dosage of NaOH in methanol solution, mainly due to the higher removal rate of lignin. Interestingly, there was no structural transformation of crystalline cellulose due to the pretreatment, which was also reflected by the FTIR results.

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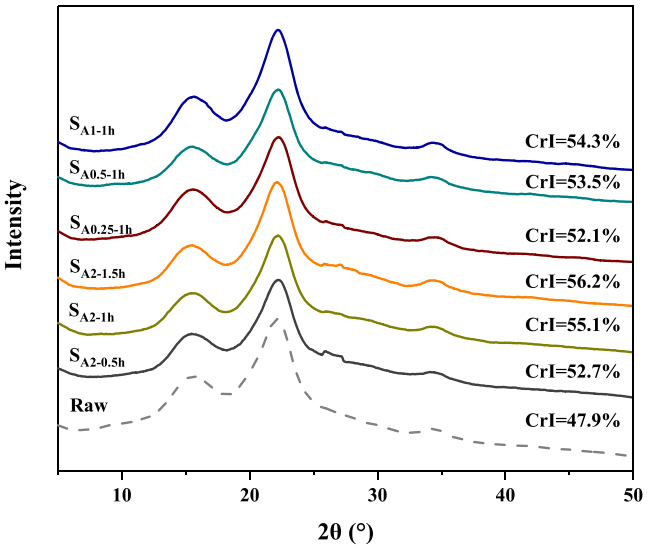

Figure 3. XRD patterns of raw material (Raw) and the extracted cellulose fractions (*S*) at different methanol pretreatments.

3.2.3. SEM Analysis. The surface morphology changes of raw material (Raw) and pretreated cellulose residues (*S*) are shown in Figure 4. The raw material exhibits a wrinkled surface with voids due to the acid hydrolysis pretreatment in the xylose production process. There are wrinkles, cracks, and holes in $S_{A2-0.5h}$ too. The surface appears to gradually dismember with the reaction time, so that more surface area could be exposed with increased sites for enzymatic hydrolysis. In samples $S_{A0.25-1h}$, $S_{A0.\ 5-1h}$, S_{A1-1h}, and S_{A2-1h}, dense holes and broken surfaces are noticed. Indeed, due to the removal of a large amount of lignin, alkali-catalyzed pretreatment results in structural changes on the biomass surface that facilitates enzyme erosion.[40,41]

3.2.4. Enzymatic Hydrolysis. The main purpose of the pretreatment was to improve the efficiency of biomass conversion to value-added products (e.g., glucose). Enzymatic hydrolysis of raw and pretreated xylose residue is compared in Figure 5 along with the glucose yield. The enzymatic hydrolysis

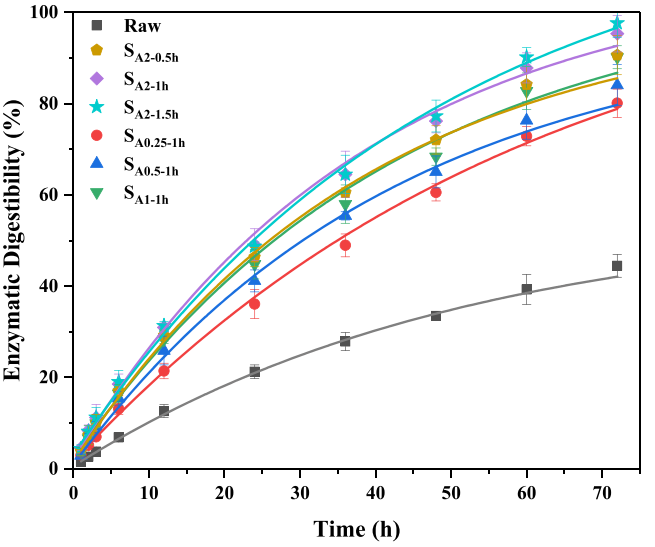

Figure 5. Glucose yield of enzymatic hydrolysis on the raw material (Raw) and extracted cellulose fractions (*S*) at different methanol pretreatments.

of raw xylose residue (Raw) produces a relatively low sugar yield (44.5%) after 72 h of hydrolysis, whereas an equal level could be accomplished with only 20−30 h hydrolysis of the pretreated xylose residue (*S*). In addition, 80.1−97.7% of the glucose yield is observed at 72 h, which indicates that *S* from the pretreatment

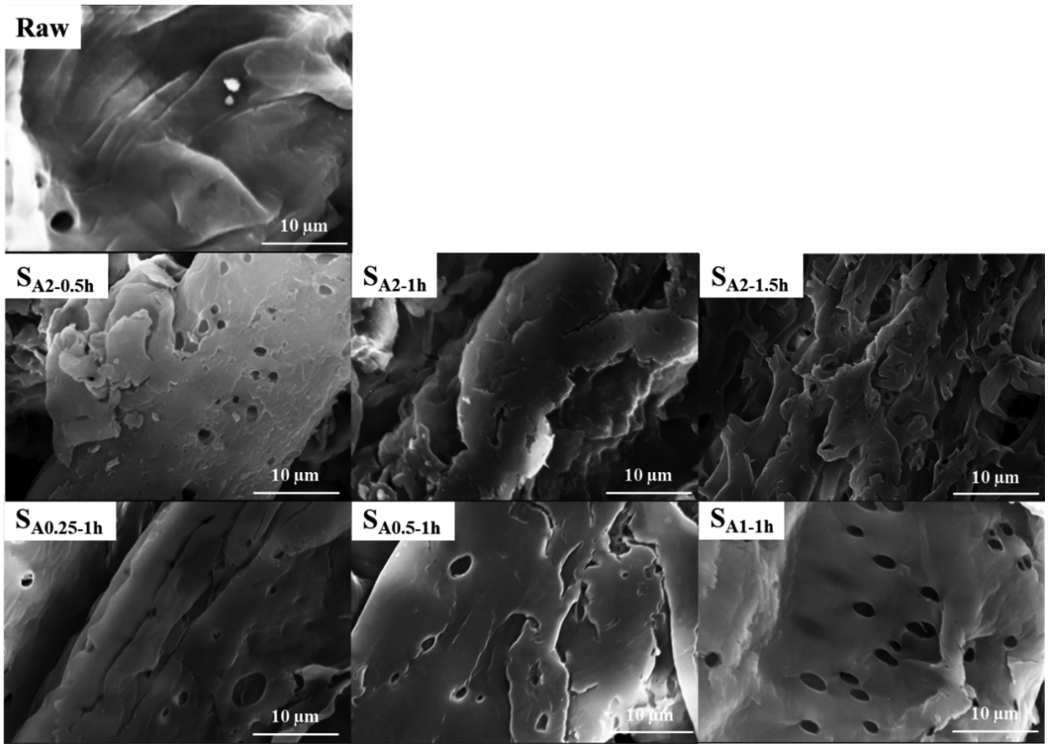

Figure 4. SEM images of raw material (Raw) and the extracted cellulose fractions (*S*) at different methanol pretreatments.

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Table 1. Sugar Content and Molecular Weight of the Lignin Fractions Extracted from Xylose Residues

	sugar content[a] (%)								
samples	Ara	Gal	Glu	Xyl	Man	Tot[b]	M_w	M_n	PDI
$L_{A2-0.5h}$	0.35	0.18	1.02	0.32	0.00	1.86	3550	2045	1.74
L_{A2-1h}	0.41	0.16	1.26	0.34	0.00	2.17	3088	1772	1.74
$L_{A2-1.5h}$	0.48	0.32	1.65	0.21	0.07	2.74	3400	1952	1.74
$L_{A0.25-1h}$	0.47	0.27	1.49	0.42	0.05	2.70	4163	2369	1.76
$L_{A0.5-1h}$	0.47	0.25	0.98	0.35	0.03	2.09	3854	2122	1.82
L_{A1-1h}	0.36	0.20	1.03	0.29	0.02	1.90	3265	1866	1.75

[a]Ara: arabinose; gal: galactose; glu: glucose; xyl: xylose; man: mannose. [b]Tot: represents total carbohydrates in the lignin samples.

is viable to biodegradation. The observed change could be due to the nonproductive adsorption of lignin on to the enzyme, resulting in the formation of lignin−enzyme complexes that retorts enzymatic hydrolysis.[42] Hence, it could be argued that cellulose residues with relatively low lignin contents could be suited best for enzymatic digestibility. Interestingly, glucose yield enhances from 90.4 to 97.7% with time from 0.5 to 1.5 h and from 80.1 to 95.3% with the NaOH dosage from 0.25 to 2%. Thus, the degradation of lignin appears to facilitate more active sites for cellulase and increases the cellulose accessibility, which is in agreement with XRD (3.2.2) and SEM (3.2.3) results. Overall, superior enzymatic hydrolysis capacity of the cellulose residue reflects its potential in bioethanol production.

3.3. Characteristics of Lignins. *3.3.1. Associated Carbohydrate and Molecular Weight Analysis of Lignins.* Due to the complex linkages between lignin and carbohydrates, the extracted lignin usually carries some amount of sugars. Herein, the recovered six lignins contained as low as 1.86 to 2.70% of sugars (Table 1). The major sugars are glucose, xylose, arabinose, and galactose along with minor amounts of mannose. Thus, these highly pure lignin samples could be conducive to future applications without further processing and certainly provide a more meaningful reference for subsequent structural property detection; and further research is warranted.

The molecular weight distribution (M_w, M_n, and PDI) of the lignin samples was determined by the GPC analysis (Table 1). It could be seen that the lignin fractions obtained by alkali-aided methanol/water system pretreatment had low M_w values, which were 1/2−2/3 of the MWL (6121 g/mol) of the same material.[43] For example, The M_w and M_n of lignin fractions from 2% NaOH−methanol/water solvent treatment for different times are in the ranges of 3088−3550 and 1772−2045 g/mol, respectively. With the increment of NaOH dosage from 0.25 to 2% (treatment for 1 h), the M_w and M_n of lignin fractions decrease from 4163 to 3088 and from 2369 to 1772 g/mol, respectively. The alkaline catalyst-assisted organic solvent appears to cut off the alkali-sensitive bonds between lignin units or between lignin and carbohydrates and effectively reduce the size of lignin molecules.[17] Interestingly, the molecular weight of lignin fractions was increased slightly from 3088 to 3400 g/mol with the treatment time from 1 to 1.5 h. The increase could be due to repolycondensation of released lignin due to prolonged processing times.[20] The lignin polydispersity (PDI) of 1.8 is smaller than the alkali and organosolv lignin of 3.64−5.58 and 2.6−7.2,[44] indicating that lignin samples released from the alkaline methanol/water systems possess a relatively homogeneous molecular size.

3.3.2. Thermal Analysis. Along with understanding the structural features of lignin, its thermal properties are also crucial for the biorefinery process. Based on the cost, energy consumption, and lignin production and properties, L_{A1-1h} was selected as a representative sample. The TGA and DrTGA thermograms of L_{A1-1h} are shown in Figure 6. The initial mass loss at around 100 °C is due to a decrease in the moisture content in the lignin fraction. The mass loss gets accelerated with increasing temperature. The degradation takes place in the temperature range of 200−700 °C with a maximum at ∼360 °C. The degradation during 200−400 °C is from the fission of lignin side chains and small molecules of lignin fractions.[45,46] Severe fractures of aromatic ring, methoxy groups, and lignin internal linkages occurred at above 400 °C, resulting in the formation of lateral unsaturated chain and small molecular volatiles.[47] The char residue at 800 °C is about 38%. It appears that the L_{A1-1h} had a similar thermal performance with xylose residue milled wood lignin (MWL) on both degradation temperature range and final char residue[43] and certainly stands as a promising candidate for further chemical conversion.

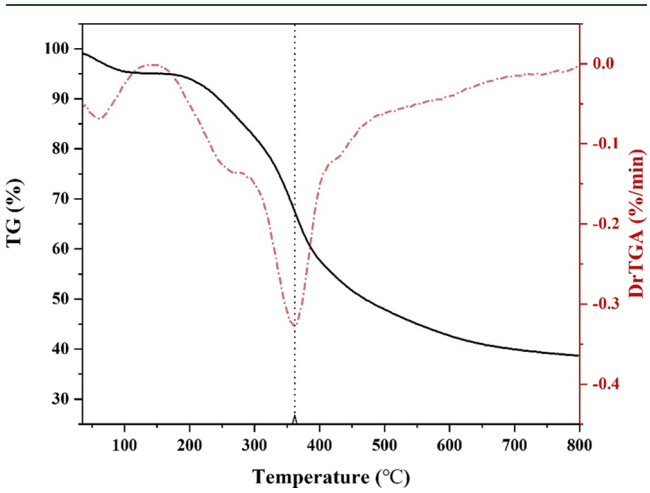

Figure 6. Thermal analyses of the extracted lignins by alkali-catalyzed methanol/water pretreatment (reaction was conducted at 80 °C for 1 h with 1 wt % NaOH dosage, L_{A1-1h}).

3.3.3. 2D HSQC NMR Analysis. The 2D HSQC NMR is a powerful elucidation technique to investigate lignin structural features, such as main chemical structures, S/G ratio, chemical linkages, and the quantitative results. Lignin is an amorphous and highly branched polyphenolic macromolecular polymer composed of three main monolignols namely *p*-hydroxyphenyl (H), guaiacyl (G), and syringyl (S) units with various ether (e.g., *β*-O-4, *α*-O-4, and 4-O-5) and C−C (e.g., *β*-*β*, *β*-1, *β*-5, and 5-5) interunit linkages.[48,49] Herein, the representative lignin L_{A1-1h} extracted from the xylose residue was analyzed. The aromatic regions (δ_C/δ_H 100−140/6.3−7.9), side chain region (δ_C/δ_H 50−85/3.1−6.2), and the major substructures of lignin fractions

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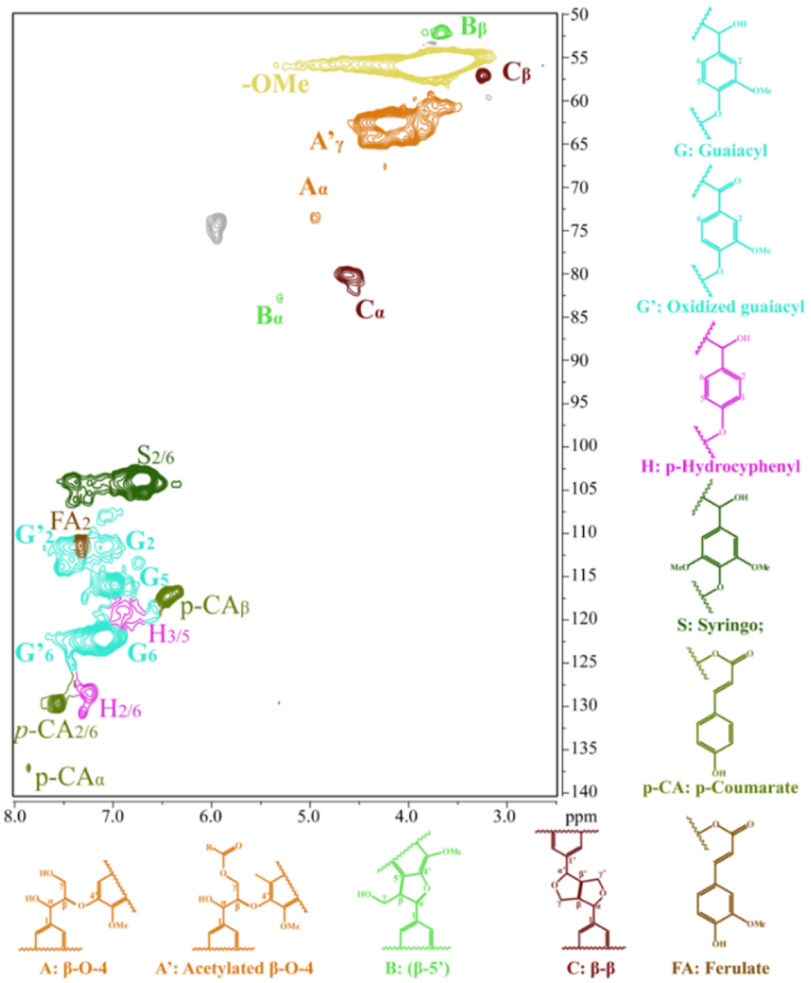

Figure 7. 2D HSQC NMR spectra of $L_{A1\text{-}1h}$

are depicted in Figure 7. The signal assignments are shown in Table S1.[50,51]

In the aliphatic region, the signals of syringyl (S), guaiacyl (G), and *p*-hydrocyphenyl (H) are clearly detected at δ_C/δ_H 103.7/6.69 ($S_{2,6}$), 110.7/7.03 (G_2), 115.0/7.01 (G_5), 118.8/6.79 (G_6), 119.8/6.98 ($H_{3,5}$), and 128.0/7.38 ($H_{2,6}$) and suggest that the obtained lignin belongs to a GSH-type lignin. The ferulate acid (FA) and *p*-coumaric acid (pCA) are seen at δ_C/δ_H 115.8/6.80 (pCA_β), 129.7/7.56 ($pCA_{2,6}$), 136.5/7.75 (pCA_α), and 111.5/7.32 (FA). In the side chain region, demethylation is not noticed in the methoxyl (OMe, δ_C/δ_H 55.8/3.73) signal. Furthermore, A_α (δ_C/δ_H 71.8/4.84), A'_γ (δ_C/δ_H 62.7/3.70−4.30), and C_β (δ_C/δ_H 52.3/3.05 are observed in the spectra. The strong signals (A) and weak signals (B and C) clearly suggest that the obtained lignin preserves the rich *β*-O-4 bonds and less *β*-*β*′ bonds. The signals of acylated *β*-O-4′ chain in *γ*-carbon (A'_γ) are also noticed. Overall, the obtained lignin preserves the sensitive *β*-O-4 linkages and shows great promise for further utilization.

3.4. Antioxidant Power Analysis. In this study, the total phenolic content (TPC), ABTS, and DPPH free radical scavenging capabilities of the lignin fractions and MWL from xylose residue are tested (Figure 8 and Table S2) and comparatively analyzed.

As phenolics are pivotal to scavenge free radicals, TPC determination before measuring the antioxidant capacity is necessary. The TPC concentration in the dried lignin sample (c_{GAE}) was expressed as the milligrams of gallic acid equivalents (GAEs) per liter of solution, and the results are shown in Figure 8a. The TPC of 299.13−361.25 mg/L obtained from the alkali-catalyzed methanol/water treatment is much higher than that of MWL of 148.76 mg/L (Table S2). The TPC increases with the treatment severity with the highest content of 361.25 mg/L for the $L_{A2-1.5h}$. In addition, a disparity in the antioxidant activity of the analyzed lignins is also observed, and the antioxidant power (AOP) of lignin fractions is found to be ∼80.0% (Figure 8b), which was much higher than that of MWL (∼50.0%, Table S2). Interestingly, there was a linear relationship between the TPC and AOP (Figure 8b), confirming that the sample with more total phenolic content possesses higher antioxidant capacity.

The free radical scavenging capacity of lignin samples is compared with a typical commercial antioxidant BHT, and the results of the DPPH assay are shown in Figure 8c. The variation of DPPH radical scavenging capacity with lignin concentration was studied for better evaluation of the antioxidant power. It was observed that the higher the lignin concentration, the higher would be the antioxidant capacity. The radical scavenging index (RSI) value of 0.79−1.24 for the obtained lignins is higher compared to 0.42 for BHT (Figure 8d) and 0.48 for MWL (Table S2). Thus, the extracted lignin had more excellent antioxidant capacity than the commercial BHT and MWL, which is in agreement with the TPC and ABTS analyses (Figure 8a,b). Thus, lignins obtained from the alkali-aided methanol/water system might make a remarkable improvement in radical

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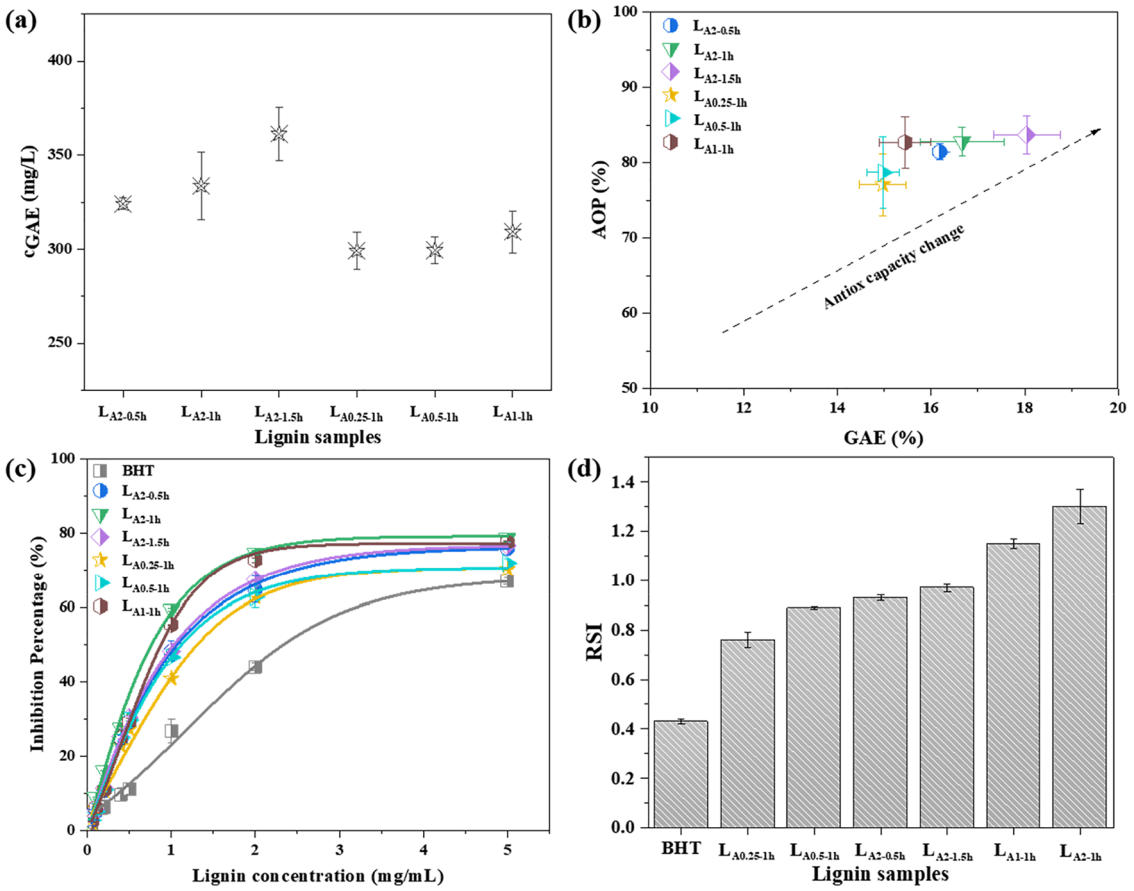

Figure 8. Antioxidant behavior of the extracted lignins through alkali-aided methanol/water system pretreatment: (a) variation of TPC (c_{GAE}); (b) correlation between AOP% and TPC% on the antioxidant capacity; (c) DPPH inhibitory effect; and (d) radical scavenging index (RSI) value.

scavenging capacity. More importantly, the Figure 8d also indicates that the DPPH radical scavenging capacity of lignins increases with a decrease in the molecular weight (Table 1) and is in agreement with the literature reports.[33,52,53] It is interesting to note that $L_{A2\text{-}1h}$ shows the highest antioxidant capacity, which is different from that of the TPC and ABTS analyses. The negative correlation between DPPH and ABTS could be due to different experimental conditions, reaction mechanisms, and other factors such as the number and position of hydrogen-donating hydroxyl groups and the presence of other proton-donating groups.[54,55] Importantly, lignin isolated from xylose residue through alkali-aided methanol/water treatment displays the highest antioxidant activity and indeed has potential for commercialization and warrants further research.

4. CONCLUSIONS

Herein, alkali-aided methanol/water pretreatment on xylose residue was demonstrated as a successful strategy to enhance the enzymatic hydrolysis and to effectively extract high-quality antioxidant lignin. The optimum pretreatment conditions (80 °C, 1.5 h, 2% NaOH) resulted in a high glucose yield of 97.7%. The recovered lignin is highly pure with low molecular weight, stable thermal behavior and high total phenolic hydroxyl content, and subtle amount of bound sugars. The results could further the production of phenolic hydroxyl of lignin with downstream antioxidant capacity of the lignin-derived materials. Overall, the designed process is an energy-saving and high conversion efficiency pretreatment system, which could provide a promising approach to obtain high value-added lignin and digestible sugars. The outcome further aids to promote whole biorefinery of lignocellulosic biomass in the future.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.energyfuels.1c00320.

Assignment of main $^{13}C-^{1}H$ cross-signals in HSQC spectra of the lignins (Table S1); and antioxidant behavior of the xylose residue MWL (Table S2) (PDF)

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Notes

The authors declare no competing financial interest.

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